



# ASSESSMENT OF SALIVARY ENZYMES- ALANINE AMINOPEPTIDASE(ALAP) AND DIPEPTIDYL PEPTIDASE IV (DPP IV) IN PATIENTS WITH CHRONIC PERIODONTITIS

**S. Rajasekar<sup>1</sup>, V. Ramasubramanian<sup>2</sup>, Sethupathi<sup>3</sup>**

<sup>1</sup>Professor of Periodontology, Rajah Muthiah Dental College & Hospital, Annamalai University, Annamalai nagar-608002, Tamil Nadu, India; <sup>2</sup>PG Student, Department of Periodontology, Rajah Muthiah Dental College & Hospital, Annamalai University, Annamalai nagar-608002, Tamil nadu, India; <sup>3</sup>Professor, Department of Biochemistry, Rajah Muthiah Medical College, Annamalai University, Annamalai nagar-608002; Tamil Nadu, India.

## ABSTRACT

**Introduction:** Alanine Aminopeptidase (ALAP) and dipeptidylpeptidase (DPP IV) are proteolytic enzymes released in the periodontal tissues from leukocytes, host cells and microorganisms. Both enzymes play an important role in collagen degradation and destruction of periodontal tissues. Therefore, detection of these enzymes might be useful in the diagnosis of periodontal disease.

**Aim:** To assess the salivary levels of ALAP and DPP IV in periodontally healthy and those with chronic Periodontitis and to correlate the enzyme levels with the severity of disease. A prospective comparative case control study was done on 60 systemically healthy patients in the age group of 25-55 and was divided into four groups with 15 patients in each as Periodontally healthy, Mild, Moderate and Severe Periodontitis. **Methodology:** Parameters like Plaque index, Gingival bleeding index, Probing pocket depth and clinical attachment loss were recorded at baseline and unstimulated whole saliva was collected from each patient and quantified for levels of ALAP and DPP IV using spectroscopic quantification method. Statistical analysis used ANOVA, Students t test and Pearson's correlation coefficient analysis was used.

**Results:** The salivary levels of ALAP and DPP IV were found to be higher in patients with Periodontitis than p healthy controls and were higher in severe ( $42.34 \pm 8.96$ ) ( $8.41 \pm 1.44$ ) and moderate group ( $25.46 \pm 6.79$ ) ( $5.17 \pm 1.31$ ) than mild Periodontitis group ( $15.83 \pm 4.54$ ) ( $3.69 \pm 1.00$ ) which was statistically significant.

**Discussion and Conclusion:** ALAP and DPP IV were higher in saliva of patients with chronic Periodontitis and positive correlation existed between the levels of these enzymes with the severity of Periodontitis.

**Key Words:** Alanine aminopeptidase (ALAP), Dipeptidylpeptidase IV (DPP IV), Saliva, Periodontitis

## INTRODUCTION

Periodontitis is a poly microbial infectious disease affecting the supporting tissues of the teeth and is a major cause of tooth loss in adults. Periodontal destruction probably results from the action of various toxic products released from specific pathogenic sub gingival plaque bacteria as well as from the host responses elicited against bacteria and their products.<sup>1</sup>

Bacterial virulence factors either result in direct degradation of host tissues or cause the release of biologic mediators from the host tissue cells that lead to host tissue destruction. Mediators produced as a part of the host response that contribute

to tissue destruction include proteinases, cytokines and prostaglandins. Another important class of molecules in tissue destruction is the variety of enzymes produced by Periodontal microorganisms such as collagen degrading enzymes, elastase like enzymes, trypsin like proteases, amino peptidases and dipeptidylpeptidases.<sup>2</sup>

Saliva is an important physiologic fluid that contains a highly complex mixture of substances. Over the last few years salivary diagnostics have received increasing attention. Saliva contains a variety of enzymes including esterases, proteinases, amino peptidases etc, synthesized and secreted by host cells and bacteria.<sup>3</sup>

### Corresponding Author:

Dr. S. Rajasekar M.D.S., Professor of Periodontology, Rajah Muthiah Dental College & Hospital, Annamalai University, Annamalai nagar-608002, Tamil Nadu, India; Contact +919443248262; Email: rsekarperio@gmail.com

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Amino peptidase is a zinc dependent enzyme produced and secreted by glands of small intestine. It helps the enzymatic digestion of proteins. Dipeptidyl peptidase IV (DPP IV) also known as adenine deaminase complexing protein 2 or CD 26 is a protein that, in humans, is encoded by the DPP IV gene. This enzyme is associated with immune regulation, signal transduction and apoptosis.<sup>4</sup>

Alanine amino peptidase (ALAP) and Dipeptidyl peptidase IV (DPP IV) activity in whole saliva of patients with Periodontitis were found to be increased when compared with healthy individuals. Both these enzymes were found to be involved in degradation of collagen.<sup>5</sup>

Thus the present study was aimed to assess the levels of Alanine amino peptidase (ALAP) and Dipeptidyl peptidase IV (DPP IV) enzymes in saliva of Periodontally healthy subjects and in patients with chronic Periodontitis and also to correlate the level of these enzymes with the severity of Periodontal disease.

## MATERIALS AND METHODS

This study was conducted in the Department of Periodontics, Rajah Muthiah Dental College & Hospital, Annamalai University, Tamil Nadu, India from July 2011 to March 2012. The study was approved by Institutional human ethical committee on 19-07-2011 and a written informed consent was obtained from all subjects participated in the study.

### Sample size determination

Sample size was determined by power analysis procedure for ANOVA test based on pilot study done 3 months before in which mean and SD for all clinical and biochemical parameters were calculated and accordingly the required sample size for each group was 15

A total of 60 systemically healthy subjects aged 25-55 were selected from the outpatient Department and divided in to four groups as ; Group 1 (Periodontally healthy) , Group 2 (Generalized chronic mild Periodontitis) , Group 3 (Generalized chronic moderate Periodontitis) and Group 4 (Generalized chronic severe Periodontitis) with 15 patients in each group. The following were the inclusion criteria for enrolment of study subjects.<sup>6</sup>

Group 1; Healthy Periodontium, absence of bleeding on probing, pockets and attachment loss.

Group 2; More than 30 % of sites with BoP , pockets  $\geq 4$  mm / CAL 1-2 mm.

Group 3 ; More than 30% of sites with BoP , pockets  $\geq 4$ mm / CAL 3-4 mm.

Group 4 ; More than 30% of sites with BoP , pockets  $\geq 5$ mm / CAL  $\geq 5$  mm.

Patients with known systemic diseases, smokers, pregnant and lactating women, those who were administered any systemic antibiotics, anti-inflammatory drugs or steroids in the preceding 3 months, and any form of periodontal therapy within the preceding 6 months were excluded from the study.

A special proforma was prepared for each subject and clinical parameters like plaque index<sup>7</sup> , Gingival bleeding index<sup>8</sup> , Probing pocket depth and Clinical attachment loss were recorded by a single examiner (SR). On the subsequent day un stimulated whole saliva was collected between 9 and 10 am by the second examiner (RS).The subject selection bias was avoided by intra examiner calibration prior to study and the investigator who collected and analysed the saliva was masked about the Group of patients as a dummy code number was given to the samples.

The subjects were first asked to rinse their mouth with distilled water to remove the food debris and saliva was collected every 60 seconds to yield a total of 5 ml of each sample in a sterile plastic container. The samples were immediately transferred to the laboratory and were centrifuged at 3000 rpm for 15 minutes.

The supernatant was collected and stored at -70°C until enzymatic assay.

Chemical reagents used for detection of ALAP

1. L-alanyl- $\beta$ -naphthylamide (1  $\mu$ mol)
2. Phosphate buffer (10  $\mu$ mol)
3. Trichloro acetic acid (40% weight/volume)
4. Sodium nitrate (0.1 % weight/volume)
5. Ammonium sulfamate (0.5% weight/volume)
6. N-(1-naphthyl)-ethylenediamine dihydrochloride (0.05% weight/volume)

Calculation formula; standard absorbent value= {optical density value optical density value} x 12.5x protein value

With sample - without sample

Chemical reagents used for detection of DPP IV

1. Glycyl-prolyl-p-nitroanilide (3Mm)
  2. Sodium hydroxide (71 Mm)
  3. Acetate buffer
- Calculation formula; optical density value (OD)  
•1.058•protein value

Chemical reagents were prepared manually for the detection of ALAP and DPP IV. ALAP was assessed by spectroscopic assay measuring the extent of hydrolysis of L-alanyl  $\beta$ -naphthylamide.<sup>9</sup>

DPP IV was assessed by spectroscopic quantification of glycyl-prolyl-p-nitroanilide hydrolysis.<sup>10</sup>

## Statistical analysis

The data obtained were subjected to statistical analysis with systat 12 software programme using ANOVA, Student t test and Pearson correlation co efficient analysis and the results were plotted in the form of tables.

## RESULTS

The Result was as following

ANOVA test comparing age range of patients revealed no significant difference within the test groups (P=0.08)

ANOVA test comparing the ALAP enzyme levels (table 2) revealed significant difference in the groups studied (P=0.01). Student t test process was applied to compare the levels of ALAP enzymes within the groups and it was observed that the enzyme level in saliva was significantly higher in test group than the controls and within the test group the severe Periodontitis group (Group 4) exhibited highest values ( $42.34 \pm 8.96$ ) followed by the moderate Periodontitis (Group 3) ( $25.46 \pm 6.79$ ) and mild Periodontitis (Group 2) ( $15.83 \pm 4.54$ ) and this was statistically significant (P=0.01).

ANOVA test comparing the DPP IV enzyme levels (table 3) revealed significant difference in the groups studied (P=0.01). Student t test process was applied to compare the levels of DPP IV enzymes within the groups and it was observed that the enzyme level in saliva was significantly higher in test group than the controls and within the test group the severe Periodontitis group (Group 4) exhibited highest values ( $8.41 \pm 1.44$ ) followed by moderate Periodontitis group (Group 3) ( $5.17 \pm 1.31$ ) and mild Periodontitis group (Group 2) ( $3.69 \pm 1.00$ ) and this was statistically significant (P=0.01).

## DISCUSSION

Chronic Periodontitis is an inflammatory condition affecting the tissues surrounding and supporting the teeth. As a result of inflammatory process, Proteolytic enzymes are released in the periodontal tissues from inflammatory cells. In addition, collagen degrading enzymes, trypsin like proteases, aminopeptidases and dipeptidylpeptidases may be produced by microorganisms. These proteolytic enzymes have an important role in periodontal destruction. Therefore, detection of these enzymes in saliva and GCF may help in periodontal diagnosis as well as to assess the progression of disease.

Our study consisted of 60 subjects with age ranging from 25-55 years and the patients were divided into four group's namely Periodontally healthy controls (Group 1), Mild Periodontitis (Group 2), Moderate Periodontitis (Group 3) and Severe Periodontitis (Group 4) with 15 subjects in each

group.

To the best of our knowledge this is the first study attempting to assess and compare ALAP and DPP IV enzyme levels in saliva of patients with mild, moderate and severe Periodontitis with respect to healthy controls.

The subjects enrolled in this study were in the age group between 25-55 years and the mean age of patients in the test group were 36.53, 40.47, and 43.13 for mild, moderate and severe Periodontitis respectively. In the present study we categorized the test group patients as mild, moderate and severe based on the stage of their disease and to the best of our knowledge this was the first such design and this helps to assess the progression of the disease with respect to the level of enzymes. Also saliva was chosen as a medium to study the enzyme activity as it has a good potential as diagnostic marker and the collection involves non invasive procedure.

ALAP is a proteolytic enzyme that plays a role in peptide hydrolysis and collagen degradation. Periodontitis is known to be a chronic inflammatory process that is characterized by an immune response that results in the release of cytokines which trigger PMNs and macrophages. Macrophages have been found to produce ALAP, while cytokines to stimulate ALAP activity. Thus several factors might be responsible for the high salivary expression of ALAP in Periodontitis.

The results of our study revealed significantly higher levels of ALAP enzyme in saliva of Periodontitis subjects when compared with healthy controls. This observation of our study was in accordance with Serenay Elgum et.al 2000<sup>2</sup>.

DPP IV is a peptidyl peptidase which seems to be involved in collagen degradation. Protease activity was found to be partly associated with inflammatory cells while DPP IV was localized in T lymphocytes and macrophages<sup>5</sup>.

In our study we observed significantly higher levels of DPP IV in saliva of Periodontitis subjects than healthy controls (p=0.01) and this was similar to the findings of Serenay Elgum et.al 2000<sup>2</sup>. Who in their study found elevated levels of DPP IV enzyme in saliva of chronic Periodontitis patients as compared to periodontally healthy controls.

In studies done by Eley BM et al <sup>11</sup> and Abiko et al <sup>12</sup> the percentage of sites positive for P.gingivalis was positively correlated with DPP IV activity. However, we in our study did not correlate the enzyme activities with sites for P.gingivalis.

In the present study we observed that the ALAP and DPP IV enzyme levels were greater as the severity of the disease increased. The enzyme levels were significantly higher in severe Periodontitis followed by moderate Periodontitis when compared to the mild Periodontitis group (p=0.01).

## Limitations

The limitation of our study was not correlating the enzyme level with the microbial load and this along with long term interventional study can be a future direction of Research.

## CONCLUSION

The following conclusions were drawn from the present study;

1. The salivary levels of enzymes Alanine amino peptidase (ALAP) and Dipeptidyl peptidase IV (DPP IV) were significantly higher in Periodontitis group as compared to healthy controls.
  2. In Periodontitis patients, there was a positive correlation between the salivary levels of enzymes ALAP and DPP IV and the severity of the disease.
- The results of our study suggest that the ALAP and DPP IV enzyme levels in saliva could reflect the extent of disease progression and hence these enzymes can be very useful biomarkers in diagnosis and treatment planning. However, further longitudinal and interventional studies are needed to validate our findings.

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**Conflicts of interest-** Nil

## KEY POINTS

Periodontitis is a chronic microbial and inflammatory disease. Various enzymes and cytokines are elevated in the tissues during the progression of the disease and these molecules are useful biomarkers of the disease. Saliva is a useful diagnostic tool for estimation of these biomarkers. In chronic inflammatory conditions like Periodontitis, proteolytic enzymes like ALAP and DPP IV are released from leukocytes and bacteria into the tissues and are responsible for collagen degradation. Estimation of these enzymes in saliva helps to assess the progression of the disease.

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**Table 1 A: Distribution of age in the groups studied**

| Groups         | N              | Mean  | S.D  | Minimum | Maximum |
|----------------|----------------|-------|------|---------|---------|
| 1. healthy     | 15             | 28.40 | 2.72 | 25      | 35      |
| 2. mild        | 15             | 36.43 | 8.12 | 27      | 52      |
| 3. moderate    | 15             | 40.47 | 7.53 | 30      | 52      |
| 4. severe      | 15             | 43.13 | 6.77 | 34      | 55      |
| <b>F value</b> | <b>P value</b> |       |      |         |         |
| 14.06          | 0.01(S)        |       |      |         |         |

**Table 1 B: Distribution of age in test groups studied**

| Groups      | N  | Mean  | S.D  | Minimum | Maximum | F value | P value  |
|-------------|----|-------|------|---------|---------|---------|----------|
| 1. mild     | 15 | 36.53 | 8.12 | 27      | 52      | 2.67    | 0.08(NS) |
| 2. moderate | 15 | 40.47 | 7.53 | 30      | 52      |         |          |
| 3. severe   | 15 | 43.13 | 6.77 | 34      | 55      |         |          |

ANOVA test comparing age range of patients between healthy controls and test groups revealed a significant difference (Table 1 A). However, there was no significant difference in the age groups of patients within the test groups (Table 1 B)

**Table 2: Comparison of salivary alanine aminopeptidase (ALAP  $\mu$ lu/mg) in the groups studied**

| Groups         | N             | Mean  | S.D  | Minimum        | Maximum        |
|----------------|---------------|-------|------|----------------|----------------|
| 1. healthy     | 15            | 3.20  | 1.12 | 1.46           | 5.49           |
| 2. mild        | 15            | 15.83 | 4.54 | 8.43           | 24.01          |
| 3. moderate    | 15            | 25.46 | 6.79 | 13.73          | 34.96          |
| 4. severe      | 15            | 42.34 | 8.96 | 25.37          | 55.65          |
| <b>F value</b> | <b>110.11</b> |       |      | <b>P value</b> | <b>0.01(s)</b> |

| Comparison | T value | P value |
|------------|---------|---------|
| 1 x 2      | 10.43   | 0.01(s) |
| 1 x 3      | 12.52   | 0.01(s) |
| 1 x 4      | 16.78   | 0.01(s) |
| 2 x 3      | 4.56    | 0.01(s) |
| 2 x 4      | 10.21   | 0.01(s) |
| 3 x 4      | 5.81    | 0.01(s) |

ANOVA test revealed significant difference in salivary alanine aminopeptidase enzyme in the groups studied( $p=0.01$ ) with higher values in test groups than healthy control.

Student t test was applied to compare the level of salivary ALAP levels within groups and the following observations were drawn;

- Groups 2,3, and 4 had higher salivary ALAP levels when compared to Group 1 with t value 10.43,12.52 and 16.78 respectively.
- Group 3 and 4 had higher salivary ALAP levels when compared to Group 2 with t value 4.56 and 10.21 respectively.
- Group 4 had higher salivary ALAP levels when compared to Group 3 with t value 5.81.



**Table 3: Comparison of salivary Dipeptidyl peptidase IV( $\mu\text{l u/mg of protein}$ ) in the groups studied**

| Groups         | N  | Mean             | S D  | Minimum | Maximum |
|----------------|----|------------------|------|---------|---------|
| 1. healthy     | 15 | 1.31             | 0.50 | 0.63    | 2.07    |
| 2. mild        | 15 | 3.69             | 1.00 | 2.05    | 5.62    |
| 3. moderate    | 15 | 5.17             | 1.31 | 2.94    | 7.25    |
| 4. severe      | 15 | 8.41             | 1.44 | 5.46    | 10.54   |
| F value 104.04 |    | P value 0.01 (s) |      |         |         |

| Comparison | T value | P value  |
|------------|---------|----------|
| 1 x 2      | 8.18    | 0.01(s)  |
| 1 x 3      | 10.62   | 0.01(s)  |
| 1 x 4      | 17.93   | 0.01(s)  |
| 2 x 3      | 3.45    | 0.02(NS) |
| 2 x 4      | 10.36   | 0.01(s)  |
| 3 x 4      | 6.42    | 0.01(s)  |

ANOVA test revealed significant difference in salivary dipeptidyl peptidase IV enzyme in the groups studied ( $p=0.01$ ) with higher values in test groups than healthy control.

Student t test was applied to compare the level of salivary DPP IV within groups and following observations were drawn;

- Group 2, 3 and 4 had higher salivary DPP IV levels when compared to group 1 with t values of 8.18, 10.62, and 17.93 respectively.
- Group 3 and 4 had higher salivary DPP IV levels when compared to group 2 with t values of 3.45 and 10.36 respectively. There was no significant difference between groups 2 and 3.
- Group 4 had higher salivary DPP IV levels when compared to group 3 with t value of 6.42